

ImageStream® Endosome/Lysosome Staining Protocol in pDC

Probing for Co-localization to Endosomes & Lysosomes

Samples: (2 x 10⁶ cells per test)

Single fluorescent color control samples – unstained, CD71 or CD107a FITC, etc

Experimental samples – untreated, positive control treatment, experimental treatment

Materials:

1. FITC anti-CD71 (BD Pharmingen cat#555536 for FITC anti-human)
2. FITC anti-CD107a (Lamp-1) (BD Pharmingen cat#555800 for FITC anti-human)
3. BDCA2-PE (Miltenyi Biotech cat#130-090-511 for PE anti-human).
4. BDCA4-PE (Miltenyi Biotech cat#130-090-533 for PE anti-human)
5. 1% paraformaldehyde in PBS (Stock 10% Formaldehyde from Polysciences cat# 04018)
6. Wash buffer (2% FBS in PBS)
7. Microcentrifuge tubes, siliconized (Sigma T4816 for 1.5mL; Sigma T4691 for 0.6mL)

Cell preparation

pDC will be isolated from PBMC according to the manufacturer's instructions (Miltenyi Biotech) .

Staining protocol

Staining done in 100 uL volumes in 1.5mL siliconized microcentrifuge tubes (DO NOT USE POLYSTYRENE TUBES). Washes are 10X volume, done at 300 x g 10' 4° C in a swinging bucket rotor.

1. Incubate cells with particle at 37 C (or 30' 4 C for negative control)
2. Wash with 0.1% BSA in PBS
3. Stain cells with surface markers (BDCA2-PE and BDCA4-PE) for 20' 4° C
4. Wash with Wash buffer
5. Fix with 1% paraformaldehyde 10' RT.
6. Wash with Wash buffer
7. Permeabilize cells with 500µL of Perm buffer (0.5% Saponin in wash buffer) for 15' RT.
8. Centrifuge to pellet and resuspend in 100µL remaining perm buffer.
9. Add 5µL heat inactivated human serum and 10µL anti-CD71 or anti-CD107 for 30' RT.
10. Wash cells with Wash buffer.
11. Fix cells in 50µL of 1% paraformaldehyde and run in ImageStream.